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Original research article

## Distribution of crayfish species in Hungarian waters

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## HIGHLIGHTS

- Occurrence of native and non-native crayfish species in Hungary in the last 20 years.
- The known distribution of *Orconectes limosus* spread further to Eastern Hungary in the last five years.
- The area occupied by *Astacus leptodactylus* shifted from the main rivers Danube and Tisza to their tributaries, because of the strong expansion of *O. limosus*.
- *Pacifastacus leniusculus* threatens the native *Astacus astacus* population in Western Hungary.

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## ABSTRACT

Three native crayfish species, i.e. *Astacus astacus*, *Astacus leptodactylus* and *Austropotamobius torrentium*, occur in Hungary. Lately, however, non-indigenous crustaceans have also invaded the country. Their most recent distribution and impact on the occurrences of the native species is not clear. Consequently, the first object of the present study was to investigate the present-day distribution and habitat preference of the native and invasive crayfish and crab species in Hungary according to water types and surface water velocity values. The second aim was to investigate the changes in the distribution patterns of all species over the last 20 years and to identify potential risks for native species in Hungary. Although *A. astacus* was discovered on several new locations the overall distribution of the species is decreasing. The rarest crayfish is *A. torrentium* which was found only at three locations, making this the most endangered crayfish in Hungary. Although *A. leptodactylus* used to occur especially in the main branches of the Danube and Tisza Rivers it shifted towards its tributaries after *O. limosus* appeared here. Despite their overlapping habitat preference and the fact that *O. limosus* is a carrier of the lethal crayfish plague, both species still co-occur at a few locations in Hungary. *Pacifastacus leniusculus* is invading the country from the West and although it is not present in large numbers yet, it has replaced the local *A. astacus* populations and may further impact its distribution when it further increases its range in the future. Although occurrences of the Chinese mitten crab (*Eriocheir sinensis*) the marbled crayfish (*Procambarus fallax* f. *virginalis*) and the red swamp crayfish (*Procambarus clarkii*) have been reported in the literature, these species were not encountered during the survey. Thus indicating that their occurrence in Hungary is not widespread yet. The increasing distribution of the invaders forms a constant threat to native crayfish populations in Hungary. To ensure the survival of the native species it is important to keep track of the ongoing changes in crayfish distributions. Nevertheless,

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additional protection measures will be required to safe-guard the survival of the native crayfish populations in Hungary.

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## 1. Introduction

The native European crayfish fauna is represented by taxa belonging to the genus *Astacus* and *Austropotamobius* (Kozák et al., 2015) whose populations are nowadays under pressure or even disappearing (Kouba et al., 2014). As one of the main causes for their decline has been put forward the arrival of crayfish originating from the North-America that carry the oomycete *Aphanomyces astacii* (Schikora, 1903) causing crayfish plague (Diéguez-Urbeondo et al., 1997). While the invaders are resistant to this disease, it is extremely lethal for the native crayfish species (Thuránszky and Forró, 1987). These invaders comprise several American crayfish species i.e., the spiny-cheek crayfish, *Orconectes limosus* (Rafinesque, 1817), the signal crayfish, *Pacifastacus leniusculus* (Dana, 1852), the marbled crayfish, *Procambarus fallax* f. *virginalis* (Martin et al., 2010), the red swamp crayfish (*Procambarus clarkii* Girard, 1852) and the catadromous Chinese mitten crab (*Eriocheir sinensis* H. Milne-Edwards, 1853) (Puky et al., 2005; Puky and Schád, 2006a,b). Although the *E. sinensis* discovered in Europe (Germany) in 1912 (Veilleux and De Lafontaine, 2007), originates from Asia it also seems to be resistant to the crayfish plague and even may act as its carrier (Schrumpf et al., 2014; Svoboda et al., 2014). Invasive crayfish species arrived in Hungary in different periods with the *O. limosus* already present for 30 years and the *P. leniusculus* being first recorded in Hungary in 1998 (Kovács et al., 2005). More recent arrivals comprise *P. fallax* f. *virginalis* and *P. clarkii*. For instance, *P. fallax* f. *virginalis* was caught in 2013 in Páhoki-canalbelt (Kovács et al., 2015), in several waters in the West Balaton region in 2014 (Lökkös et al., 2016), and in warm water lakes of the capital city and at the effluent of these lakes into the Danube (Weiperth et al., 2015). On the other hand, *P. clarkii* was found in Lake Városliget were in 2015 only one specimen was caught (Weiperth et al., 2015). Both *P. fallax* f. *virginalis* and *P. clarkii* are popular in the pet trade and have established populations in nearby countries like Slovakia, Croatia, Ukraine and Austria (Samardžić et al., 2014; Keller et al., 2014; Novitsky and Son, 2016; Lipták et al., 2016; Petutschnig, 2008). These invasive species may colonize new areas quickly because they spread actively through canals and rivers and passively during floods and through human activities (Holdich and Pöckl, 2007). According to Bernardo et al. (2011), the colonization rate in the river Maças (Portugal) by *P. leniusculus* ranged from 0.8 to 2.6 km yr<sup>-1</sup>. In contrast, the downstream dispersal rate of *O. limosus* in the Mura river, Croatia, was 18–24.4 km yr<sup>-1</sup> (Hudina et al., 2009), while a rate of 13 km yr<sup>-1</sup> has been observed in the River Danube in Hungary (Puky and Schád, 2006b). This latter river used to be inhabited by *Astacus astacus* in the 1990s, but it is now dominated by *O. limosus* (Holdich and Pöckl, 2007).

Despite the arrival of the invasive crustacean species, the three native species, the noble crayfish *Astacus astacus* (Linnaeus, 1758), the narrow-clawed crayfish *Astacus leptodactylus* (Eschscholtz, 1823) and the stone crayfish *Austropotamobius torrentium* (Schrank, 1803) are still present in Hungary (Puky et al., 2005). However, their current distribution is unclear since the last comprehensive overview of crayfish occurrences in Hungary published in 2013 (Györe et al.) was mainly based on literature data containing observations till 2006 for native species and observations till 2012 for non-native species. In addition, the overview of Györe et al. (2013) presented information on crayfish distribution in large sized grids of 50 km and consequently lacked details on for instance crayfish occurrence per water type. Our present investigation contains data from very detailed field monitoring studies spanning more than two decades, from 1995 up to 2016 and includes exact geographical positions of the sampled sites together with information on environmental conditions (e.g., water type, dimensions of waterbodies, water flow). The aim of this present study is two-fold. Firstly it aims to investigate the present-day distribution and habitat preference of the native crayfish species *A. astacus*, *A. leptodactylus* and *A. torrentium* and the distribution of the invasive crayfish and crab species *O. limosus*, *P. leniusculus*, *P. fallax* f. *virginalis*, *P. clarkii* and *E. chinensis*. Secondly it aimed to investigate changes in the distribution patterns of all species over the last 20 years to identify potential risks for native species in Hungary.

## 2. Material and method

### 2.1. Sampling

For our research we used existing data from different short- and long-term projects between 1995 and 2015 (BioAqua Pro Ltd., <http://www.bioaquapro.hu/hu/referenciak>). In total 1268 water systems comprising 4043 sampling sites have been sampled.

Crayfish were sampled according to the ‘multi habitat sampling’ procedure for macro-invertebrates (Juhász et al., 2009). Different microhabitats were sampled in proportion to their percentage presence which was determined prior to the sampling. The “kick and sweep” technique (Juhász et al., 2009) with a standard dip net (with a 950 µm mesh fabric and a 25 × 25 cm metal frame) was applied to collect the animals. Complementing the sampling, additional qualitative (faunistic) samples were taken following Nieuwenhuis (2005).

Each sampling location was categorized as being ‘lowland’ or ‘upland-hilly’ and ‘calic’ or ‘siliceous’ type. Furthermore, the size of the waterbody was categorized as ‘extra small’, ‘small’, ‘medium’, ‘large’ and ‘extra large’, following the Water Framework Directive water body typology. Furthermore, all standing water bodies were assigned to the category ‘lakes’. In Hungary, extra large lakes do not occur, so these kinds of waters were not included in the database. In addition, the surface water velocity was estimated by measuring the distance travelled of a plastic cap in 10 s.

## 2.2. Data analysis

Potential trends in the crayfish catches (e.g. increase or decrease of crayfish abundance) were determined for the period between 1995 and 2015. Quantum GIS Wien 2.8.1 software ([www.qgis.org](http://www.qgis.org)) was used to map the distribution of the crayfish species. To obtain insight in changes in crayfish distributions over time, the observations were divided into four time periods: from 1995 to 2000, from 2000 to 2005, from 2005 to 2010 and after 2010. Comparing the distributions between these periods showed either a decreasing, stable or increasing range per species.

We counted the number of crayfish observations in the different types of waterbody categories. Additionally, the average abundance was calculated per species per water body type. Finally, crayfish occurrence in relation to surface water velocity was also calculated.

## 3. Results

### 3.1. Geographical distribution

*Astacus astacus* was found in 68 sites from 40 watercourses and is currently mostly found in the South-Western and Northern part of the country (Fig. 1). Note that especially between 2000 and 2015 additional habitats of *A. astacus* were discovered mainly in the northern, southern and western part of the country (Fig. A.1).

*A. leptodactylus* occurred in 102 sites from 31 watercourses where the species mostly occurred in the main stream of the Danube and Tisza rivers their backwaters and in smaller sized rivers connected to these main streams (Fig. 1). Note that before the year 2000, the Tisza River was the main area where this crayfish occurred; Between 2000 and 2010, the species still occurred here and maintained populations in the upstream section of the Tisza River and in the Danube and in its tributaries as well. From 2010 onwards, however, the occurrence of this species decreased here and shifted from the main branches of the Tisza to its tributaries, channels and smaller streams (Fig. A.1).

The distribution of *A. torrentium* is limited to small streams in the north and west of the country (Fig. 1 and Fig. A.1). It was only encountered at 3 sampling locations and consequently this species can be considered very rare in Hungary.

*O. limosus* was found in 90 sites from 39 watercourses. This species seems nowadays the most widespread invasive crayfish species in Hungary. Before 2005 only 3 observations from the Danube river system were known, but the distribution area of this species increased rapidly thereafter since *O. limosus* colonized backwaters, tributaries and channels connected to Danube and Tisza-lake reservoir. After 2010, the species colonized other watercourses with a connection to Danube and Tisza as well (Fig. 1 and Fig. A.1).

The other invasive crayfish, *P. leniusculus*, occurred in 20 sites from 6 watercourses only (Fig. 1 and Fig. A.1). Between 1995 and 2005, the species was found only in one sampling site and in the period 2005–2010, the species increased its range but is nevertheless still limited to the western part of the country.

In our monitoring we did not find any specimen of the *E. sinensis*, *P. fallax* f. *virginalis* and *P. clarkii*.

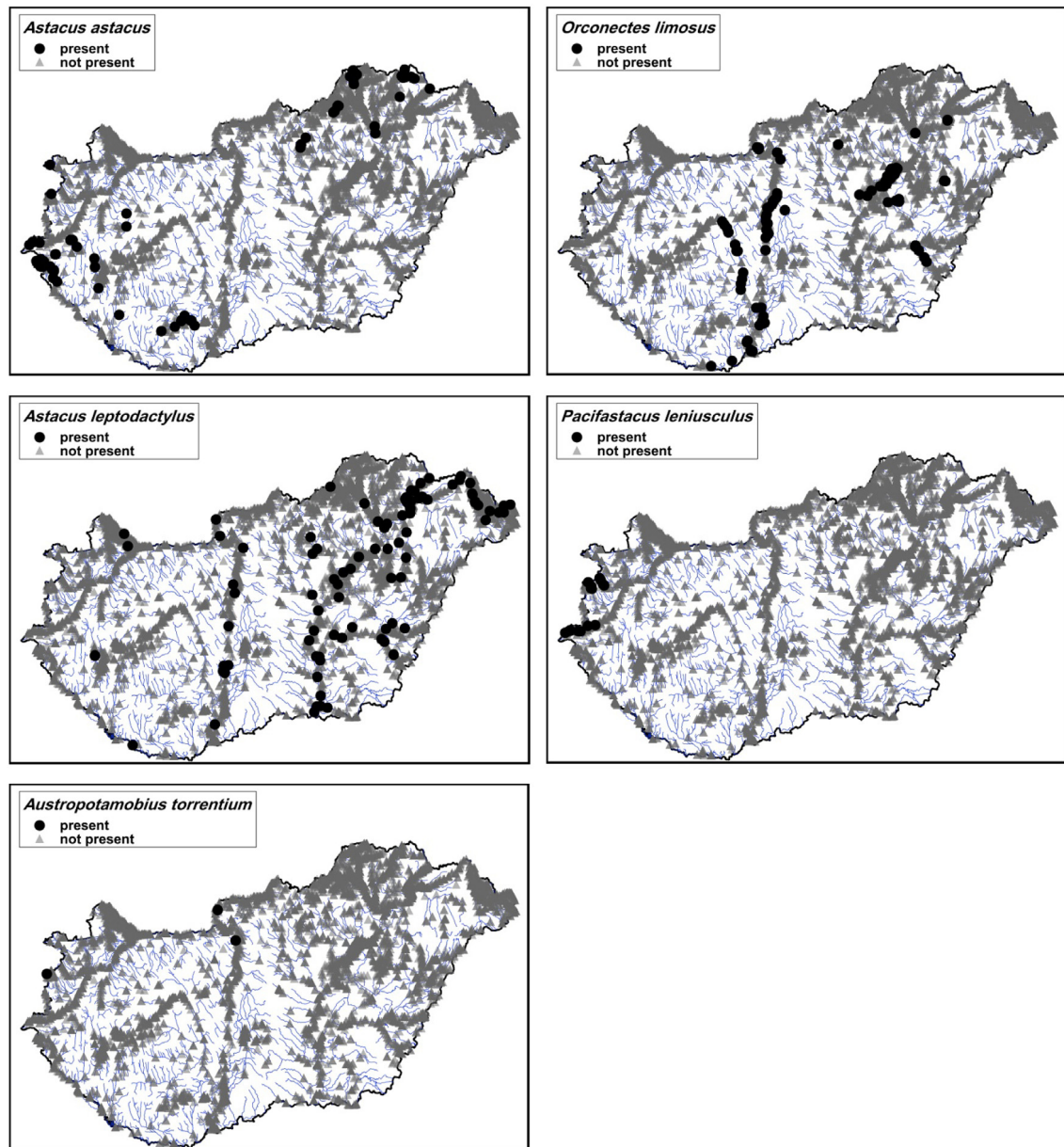
### 3.2. Coexistence

According to the data of the last two decades, both coexistence with and the outcompeting of native crayfish species by invaders occur in Hungary (Table 1). Coexistence of native species occurred only in one sampling site (*A. leptodactylus* and *A. astacus*); no evidence was found of one species outcompeting the other. The presence of invasive and native species on the same habitat was more common. *O. limosus* co-occurred with *A. leptodactylus* on 5 sampling sites and only on one site with *A. astacus*, while *P. leniusculus* was found in one sampling site together with *A. astacus*. On four other sites it was observed that invasive species were indeed outcompeting the native ones, since on 3 sites *O. limosus* replaced *A. leptodactylus* and on one site *P. leniusculus* replaced *A. astacus*.

**Table 1**

Number of sites where coexistence (with light grey background) and outcompeting (with dark grey background) occurs with different crayfish species in Hungary.

	<i>A. astacus</i>	<i>A. leptodactylus</i>	<i>A. torrentium</i>	<i>O. limosus</i>	<i>P. leniusculus</i>
<i>A. astacus</i>	—	1	0	1	1
<i>A. leptodactylus</i>	0	—	0	5	0
<i>A. torrentium</i>	0	0	—	0	0
<i>O. limosus</i>	0	3	0	—	0
<i>P. leniusculus</i>	1	0	0	0	—



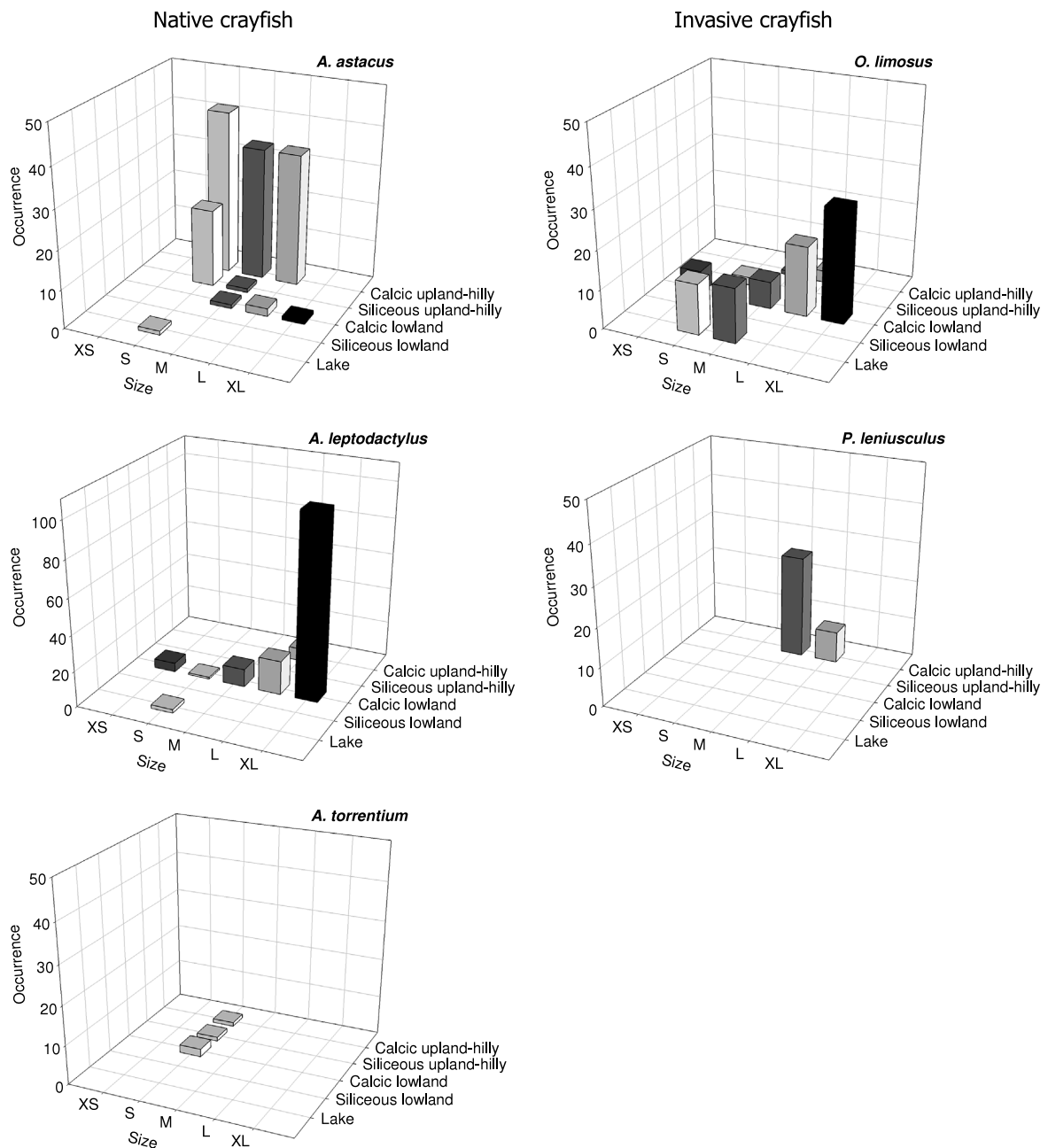
**Fig. 1.** Distribution area of the different crayfish species in Hungary (black dots) from 1995 till 2015 (Grey triangles indicate sampled sites).

### 3.3. Environmental characteristics

*Astacus astacus* was mainly found in upland–hilly water bodies and occurred in small, medium and larger sized water bodies. Although also found in other types of waters, *A. astacus* more frequently occurred in calcic waters, (Fig. 2). Despite this observation the average abundance was the highest in small sized siliceous, upland–hilly waters with  $18.4 \pm 54$  individuals/m<sup>2</sup> (Table 2). The total number of individuals of *A. astacus* was the highest on sampling sites with water velocities between 0.11 and 0.30 m s<sup>−1</sup>, with its absolute peak between 0.25 and 0.30 m s<sup>−1</sup>. At higher velocities, *A. astacus* abundance decreased rapidly (Fig. 3).

In contrast to *A. astacus*, the *A. leptodactylus* was found mainly in calcic larger sized lowland streams, i.e., large and extra large water bodies (Fig. 2). The highest average abundance of  $43 \pm 1.2$  individuals m<sup>−2</sup> was observed in the calcic, lowland and extra large waters (Table 2). Most individuals of *A. leptodactylus* were captured in slower moving waters with water velocities between 0.06 and 0.1 m s<sup>−1</sup> (Fig. 3).





**Fig. 2.** Frequency of occurrences of the crayfish species in different water type categories. Note that occurrence in a certain water type indicates the amount of sites with this specific species.

The *A. torrentium* seems to inhabit both calcic and siliceous smaller streams (Fig. 2). Moreover, the average abundance was low with  $0.5 \pm 1.6$  individuals  $m^{-2}$  (Table 2).

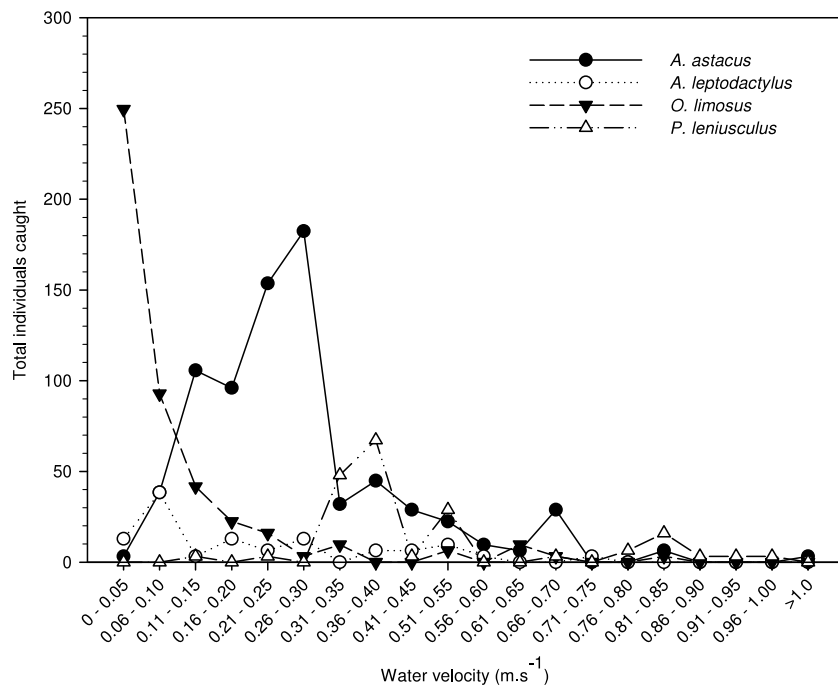
The most widespread invasive crayfish in Hungary is *O. limosus* occurring both in streams and lakes. Although the species occurred in various water types it was captured more frequently in calcic, larger sized lowland waters (Fig. 2). The highest abundance was found in both calcic, lowland small ( $11.2 \pm 4.8$  individuals  $m^{-2}$ ) and large streams ( $11.4 \pm 3.1$  individuals  $m^{-2}$ ) (Table 2). *O. limosus* was mostly captured at sites with a lower surface water velocity between 0 and  $0.1 m s^{-1}$  (Fig. 3).

The *P. leniusculus* occurred in a very limited number of habitats restricted to the calcic, upland and hilly, medium and large sized water systems (Fig. 2). The population abundance in these waters was  $16.3 \pm 12.0$  individuals  $m^{-2}$ , while in large waters this value was only  $4.8 \pm 0.7$  individuals  $m^{-2}$  (Table 2). *P. leniusculus* was caught in watercourses with a surface water velocity between  $0.3$  and  $0.4 m s^{-1}$  (Fig. 3).

**Table 2**

Average population density values of crayfish species in the different water type categories (P: presence of the crayfish; no quantitative data available).

	Size	Average abundance values $\pm$ SEM			
		Type			
		Calcic, upland–hilly	Siliceous, upland–hilly	Calcic, lowland	Lake
<i>A. astacus</i>	XS	–	–	–	–
	S	14.9 $\pm$ 3.08	18.4 $\pm$ 5.4	–	0.6 $\pm$ 0.6
	M	14.1 $\pm$ 4.8	1.6 $\pm$ 0.5	P	–
	L	5.9 $\pm$ 1.5	–	12.8 $\pm$ 2.1	–
	XL	–	–	P	–
<i>A. leptodactylus</i>	XS	–	–	2.7 $\pm$ 0.5	–
	S	–	–	P	1.1 $\pm$ 1.1
	M	–	–	1.6 $\pm$ 0.5	–
	L	3.7 $\pm$ 1.4	–	3.4 $\pm$ 0.5	–
	XL	–	–	4.3 $\pm$ 1.2	–
<i>A. torrentium</i>	XS	–	–	–	–
	S	P	P	0.5 $\pm$ 1.6	–
	M	–	–	–	–
	L	–	–	–	–
	XL	–	–	–	–
<i>O. limosus</i>	XS	–	–	3.2 $\pm$ 0.0	–
	S	–	1.06 $\pm$ 1.06	11.2 $\pm$ 4.8	8.7 $\pm$ 3.6
	M	P	–	6.8 $\pm$ 2.1	7.2 $\pm$ 1.8
	L	6.4 $\pm$ 3.2	–	11.4 $\pm$ 3.1	–
	XL	–	–	5.5 $\pm$ 0.7	–
<i>P. leniusculus</i>	XS	–	–	–	–
	S	–	–	–	–
	M	16.3 $\pm$ 12	–	–	–
	L	4.8 $\pm$ 0.7	–	–	–
	XL	–	–	–	–

**Fig. 3.** Crayfish occurrence in relation to surface water velocity.

The most crayfish species were found in calcic, upland–hilly and in calcic, lowland waters. Siliceous, lowland waters were not sampled, because these are not present in Hungary.

#### 4. Discussion and conclusions

Due to an increased sampling strategy in the last years, *A. astacus* was found at several new locations which mainly occurred in the smaller upper sections of streams with higher surface water velocity supporting earlier observations of the distribution of this species (Schulz, 2000; Kovács et al., 2005; Horvai et al., 2010). As a result, the current distribution of *A. astacus* seems to be much wider than the distribution described previously by Puky and Schád (2006a).

In our study, *A. leptodactylus* was mostly found in the main branch of Tisza and its connected watercourses as well as in the stagnant backwaters which support the observations made by Pöckl (1999). This distribution pattern, with a few new observations in the eastern part of the country, does not deviate much from the one described by Puky and Schád (2006a). The distribution pattern, however, seemed to shift after *O. limosus* appeared in the bigger streams of Hungary. The *A. leptodactylus* disappeared from the Danube and a few of its tributaries and retreated to its channels and side branches. This phenomena does not occur in the Tisza river where the main habitats of *A. leptodactylus* occurred in the main river, while *O. limosus* occurred in the Tisza-lake reservoir and channels and side branches connected to Tisza.

*Austropotamobius torrentium* occurs only very locally in Hungary and was found only in mountain streams in the north and west of the country. Our observations support the general view that this species lives in small streams (Vlach et al., 2009) and requires pristine waters (Pârvulescu et al., 2011).

The invasive *O. limosus* is well-established in the main branches of the Danube and Tisza River their backwaters and connected channels. Our data support earlier observations that this species mainly occurs in Central and Eastern Hungary (Györe et al., 2013). However, it seems that the distribution of this species spread further eastwards of the country in the last five years (Fig. A.1). In addition, *O. limosus* expanded strongly in the Danube Basin reaching many Danube river tributaries along its colonization pathway (Lipták and Vitázková, 2014). In 2009, the species was found at the Romanian–Serbian border already approx. 600 km away from our sampling sites which were situated on the lowest downstream point in our country (Pârvulescu et al. 2009, Pârvulescu et al., 2012). *O. limosus* generally occurs in very different types of water bodies (Aklehnovich and Razlutskiy, 2013), but our study indicates that the species mostly occur under low flow conditions (Fig. 3). Interestingly, *A. leptodactylus* and *O. limosus* were co-occurring on a few sampling sites after 2003. These sites occurred in larger streams and in branches which were in direct contact with the main river. In other cases, the establishment of *O. limosus* meant that *A. leptodactylus* was replaced in a few years. This could mean that in the current sites were co-existence was observed, this might merely the onset to final replacement. The fact that replacement is not instantaneous, might be an indication that these populations of *A. leptodactylus* might be more resistant towards the crayfish plague. A possibility shown earlier in a Turkish population (Svoboda et al., 2012; Jussila et al., 2014). Molecular evidence suggests that persistent infections of less virulent strains of the crayfish plague can occur in native crayfish populations without extinction of the host (Schrimpf et al., 2013; Svoboda et al., 2012; Jussila et al., 2014). When crayfish plague is not the main impact on native populations, replacement can also be driven by the fact that *O. limosus* is more resistant to heavy pollution and low oxygen concentrations, or outcompetes competitors via its rapid population growth, high somatic growth rate, early puberty and high fecundity (Aklehnovich and Razlutskiy, 2013). In addition, also the possibility of facultative parthenogenesis has been reported (Buřič et al., 2011, 2013).

*P. leniusculus* was first observed in 1998 in the west side of the country (Kovács et al., 2005) where it probably entered Hungary through the Rába River (Bódis et al., 2012). At first the species mainly colonized smaller streams. This distribution area, however, also overlaps with that of *A. astacus*. Although, both co-existence and replacement of *A. astacus* by the signal crayfish exists only on one sampling site each, it indicates a problem, because these two species have nearly the same habitat requirements. In addition, *P. leniusculus* grows faster, has a higher reproduction rate and displays a more aggressive behaviour in competitive encounters (Westman and Savoleinen 2001; Holdich et al., 2009; Hudina et al., 2011). In all cases where both species have been observed together at the same time in the same river, only the signal crayfish survived and the noble crayfish disappeared (Pöckl, 1999). North American crayfishes, like *O. limosus* and *P. leniusculus* are carriers of the, for indigenous crayfish lethal, crayfish plague pathogen (*A. astaci*). For some non-native species, all individuals of a population are infected (Diéguez-Urbeondo et al., 1997; Söderhäll and Cerenius, 1999; Cerenius et al., 2003). When distributions of non-native and indigenous crayfish species overlap, the indigenous species become infected by this pathogen (Kozubíková et al., 2010).

The *E. sinensis* has expanded its distribution throughout northern Europe since its introduction into Europe in 1912 (Robbins et al., 2006; Schrimpf et al., 2014). The species was collected once in the main arm of the Danube in 2003 near Budapest (Puky et al., 2005) and 200 km downstream from the same river in 2004 (Puky and Schád, 2006a). Presumably it was introduced via ballast water (Robbins et al., 2006) or from an intentional introduction as in other European countries (Herborg et al., 2003), but the species has not been observed in our monitoring and neither in other studies, despite of the fact, that already known in the Austrian and Serbian Danube (Puky et al., 2005).

In 2013 and 2014, *P. fallax* f. *virginalis* was found near Lake Balaton (Kovács et al., 2015) and in waters in the West Balaton region (Lökkös et al., 2016). Another *Procambarus* species, i.e. *P. clarkii* was found in Lake Városliget in 2015 (Weipert et al., 2015). However, none of these *Procambarus* species were observed in our study and we could therefore not confirm their presence. Nevertheless, an increase of *P. fallax* f. *virginalis* populations is not unlikely, due to its parthenogenetic reproduction strategy and its overwintering ability in Central European climatic conditions (Lipták et al., 2016). The same goes for *P. clarkii*, which also has also been reported to have the potential for asexual reproduction (Buřič et al., 2011).

The habitat of *A. torrentium* does not overlap with invasive species, but this crayfish is the rarest species in Hungary and is consequently most at risk; if they come into contact with invasive species and thereby with the crayfish plague they will likely go extinct. This is corroborated by the fact that in South-Western Romania, populations of the stone crayfish recently got endangered due to further invasions of *O. limosus* from both Hungary (Tisza River) and Serbia (Tamiš River) (Lipták and Vitázková, 2014).

According to our analysis *A. leptodactylus* is at risk to be replaced by *O. limosus* in Hungary, because of an overlap in their occurrence, their habitat requirements (Fig. 2, Table 2) and preferred surface water velocity (Fig. 3). The risk on replacement for *A. astacus* is high as well because it is threatened by *P. leniusculus* in calcic, upland –hilly waters with larger sizes (Fig. 2) and in waters where surface water velocity values are higher (between 0.35 and 0.55 m s<sup>-1</sup>). However, smaller sized waters are not yet occupied by *P. leniusculus* and here *A. astacus* is less at risk for now (Figs. 2 and 3, Table 2). Maybe these small size waters can in future serve as refuges to *A. astacus* and provide a first basis in sustainable management in the conservation of noble crayfish populations.

To ensure the survival of Hungarian native crayfish, it is of great importance to keep track of the ongoing changes in the distribution area of the native as well as non-native crayfish species. The increase of non-native crayfish species is correlated with especially urbanization level and the Gross domestic product (GDP) per capita (Perdikaris et al., 2012). Where more urbanization results in a higher number of NICS in EU countries, while the number of NICS seem to increase at increasing GDP per capita. Interestingly on the later a tipping point seem to be present because the amount of NICS seems decrease in countries with a GDP higher than \$40,000 per capita. Also in Hungary, the urbanization level is well correlated with the number of NICS (Fig. A.2). The connection between capital accumulation and number of NICS is also well correlated and still increases up to now (World Bank, 2016) (Fig. A.2).

As a consequence, protection measures will be required to safe-guard the survival of the native crayfish populations. These measures could for instance comprise the construction of crayfish barriers to frustrate ongoing invasions (Kerby et al., 2005). However, such barrier can restrict the movement of aquatic wildlife (e.g., frustrating fish migration). A solution might be to construct fish-passable barriers (Frings et al., 2013) or to really fence off certain waters for crayfish protection at the expense of fish. All these should be part of an active crayfish management and protection plan supported for instance by crayfish breeding centres for establishing new or strengthen weak populations, like is present in Estonia (Paavert and Hurt, 2009).

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## Appendix A. Supplementary data

Supplementary material related to this article can be found online at <http://dx.doi.org/10.1016/j.gecco.2016.09.009>.

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